REMARKS

Claim 1-4 and 7 are pending in the application. The claims are directed to highly purified preparations of glycosylated polypeptides comprising a CD44 amino acid backbone and sialylated, fucosylated glycans and having E-selectin or L-selectin ligand activity.

The examiner maintains a series of rejections under 35 U.S.C. § 103(a) citing Sackstein 1997[1], Stamenkovic[2], and Dougherty[3] as primary references. The secondary references cited by the examiner include Ni[4], McEver[5], Lasky,[6] and Oxley.[7] These rejections, however, fail to provide a reasonable rationale or clear articulation as to how a person of ordinary skill in the art could have arrived at the claimed invention based on the teaching of these references. MPEP § 2142 summarizes the law on this point as follows (citations omitted):

> The key to supporting any rejection under 35 U.S.C. 103 is the clear articulation of the reason(s) why the claimed invention would have been obvious. The Supreme Court in *KSR International Co. v. Teleflex Inc.* noted that the analysis supporting a rejection under 35 U.S.C. 103 should be made explicit. The Federal Circuit has stated that "rejections on obviousness cannot be sustained with mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness."

The rejections set forth by the examiner have failed to meet this standard and should be withdrawn. A full analysis of Sackstein 1997, Stamenkovic, Dougherty, Ni, and McEver can be found on pages 16-29 of the Appeal Brief filed February 20, 2009. Lasky and Oxley are cited in new grounds of rejection in the Office Action of August 20, 2009 and will be addressed hereinafter. It should be noted that none of these references disclose highly purified preparations of the CD44 glycoproteins according to the present claims. Rather, it is the examiner's position that the cited references provide "for key starting materials (e.g., KG1a, Namalwa), probes to isolate CD44, CD44R1 and HCELL (e.g., L-selectin ligand probes or CD44 probes) as well as functional and structural characteristics for said molecules" in order to isolate the recited highly purified preparations of CD44 glycoproteins.[8] The analysis provided herein will demonstrate

---

1 Sackstein *et al.*, Blood. 1997 Apr 15;89(8):2773-81.
2 Stamenkovic *et al.*, EMBO J. 1991 Feb;10(2):343-8.
3 Dougherty *et al.*, J Exp Med. 1991 Jul 1;174(1):1-5.
4 U.S. Patent No. 5,942,417.
5 U.S. Patent No. 6,124,267.
6 U.S. Patent No. 5,652,343.
7 Oxley *et al.*, Blood. 1994 Nov 15;84(10):3299-306.
8 Page 4, fifth full paragraph, of the Office Action mailed August 20, 2009.

that combining prior art elements according to examiner could not predictably yield highly purified preparations of CD44 glycoproteins.

Sackstein 1997 discloses the identification of an activity of an unknown protein contained within the whole cell lysates of KG1a cells. Sackstein 1997 fails to identify the polypeptide as having a CD44 amino acid backbone, let alone purify a CD44 glycoform. Sackstein 1997 identified the existence of an unknown, non-descript molecule having L-selectin binding activity that is expressed in the human hematopoietic cell line KG1a. Sackstein 1997 does not suggest to a person of ordinary skill in the art would that the unknown molecule was CD44, let alone a CD44 glycoprotein with a CD44 amino acid backbone.

Indeed, Sackstein 1997 teaches away from CD44 as a possible candidate for the molecule displaying L-selectin activity. Sackstein 1997, in the Abstract, specifically states that the "native membrane L-selectin ligand exhibit[s] sulfate-independent function." Maiti[9], published in 1998 in the reputable journal *Science*, is offered as evidence that a person of ordinary skill in the art would have considered that sulfation was required for CD44-mediated leukocyte adhesion. Specifically, Maiti expressly teaches as follows:

> The proinflammatory cytokine tumor necrosis factor-α, but not interferon-γ, was found to convert CD44 from its inactive, nonbinding form to its active form by inducing the sulfation of CD44. This posttranslational modification was required for CD44-mediated binding to the extracellular matrix component hyaluronan and to vascular endothelial cells.[10]

In contrast, Sackstein 1997 expressly teaches that the identified L-selectin binding activity was sulfate independent.[11] That is, the molecule described in Sackstein 1997 possessed the ability to bind to L-selectin, a leukocyte glycoprotein that mediates adhesive interactions, in a reaction that did not require sulfation. As CD44 was known to be sulfated and it was thought to require sulfation to function, a person of ordinary skill in the art would have been led to believe that the molecule described by Sackstein 1997 was not CD44.

This point is bolstered by the Picker Declaration of record filed August 26, 2009. In this declaration, Louis Picker explains that CD44 is known to be sulfated, which would have led anyone knowledgeable in the field to exclude the role of CD44 as a selectin ligand. Further,

---

[9] Maiti *et al.*, "TNF-alpha induction of CD44-mediated leukocyte adhesion by sulfation," Science. 1998 Oct 30;282(5390):941-3.

[10] Matai at Abstract.

[11] Sackstein 1997 at Abstract.

Louis Picker explains that "isolated, purified CD44 was frequently used as a "negative control" by us and others to compare against authentic selectin ligands precisely because it was shown to be non-reactive with HECA-452 mAb and found to be devoid of selectin ligand activity." The Declaration of Robert Sackstein filed January 18, 2010 at paragraphs 4 and 5 (the "HECA-452 Declaration") further explains that HECA-452 mAb alone is insufficient to purify the presently claims glycoproteins.

With regard to Stamenkovic and Dougherty, neither Stamenkovic nor Dougherty identify specific glycoforms of CD44 or distinguish functional differences between CD44 glycoforms, and accordingly, could not lead one of ordinary skill in the art to a purified preparation containing CD44 glycoforms comprising sialylated, fucosylated glycans. Applicant has submitted evidence demonstrating that one of skill in the art would not have been able to predictably modify the teachings of these references. Namely, Katoh[12/] showed that the adhesive function of CD44 to its well-known ligand hyaluronic acid is actually inhibited by certain carbohydrate modifications, such as sialic acid. Thus, a CD44 glycoform comprising sialylated glycans would have not been thought to possess any adhesion specificity, let alone be identified as an E- or L- selectin ligand. The teachings of Picker,[13/] Berg,[14/] Jutila,[15/] and Walcheck[16/] each, and altogether, provide evidence for the well-established (prior) canon in the art — that is, it was generally understood in the art at the time of invention that CD44 was not a selectin ligand. This evidence has not been properly considered by the examiner.

The inappropriateness of Namalwa cells disclosed in Stamenkovic was previously addressed in the Response to Office Action submitted Sept. 27, 2004 (pp. 20-21) and in the Response to Final Office Action (pp. 17-18) and accompanied Declaration of Robert Sackstein submitted July 27, 2005. As explained in the above, it would not be possible for a skilled artisan to produce the highly purified preparations of the recited CD44 glycoforms using the Namalwa cells because these cells lack the necessary glycosyltransferases to produce the recited CD44 glycoforms. This evidence has never been specifically refuted or addressed by the examiner and has not been properly considered by the examiner.

12/ Katoh *et al.*, J Exp Med. 1995 Aug 1;182(2):419-29.
13/ Picker *et al.*, American Journal of Pathology, 136:1053-1068, 1990.
14/ Berg *et al.*, J Exp Med. 1991;174:1461-1466.
15/ Jutila *et al.*, J Immunol. 1994;153:3917-3928
16/ Walcheck *et al.*, J Exp Med. 1993 Sep 1;178(3):853-63.

As a result, there is no clear articulation on record as to how a skilled artisan would have modified the teaching of Sackstein 1997, Stamenkovic, and/or Dougherty to arrive at the claimed purification. Rather, the examiner, improperly, sustains the rejections with the mere conclusory statement that the submitted evidence "appear to ignore the relationship of the references molecules to a human hemopoietic cell line."[17/]

The examiner also relies on newly cited references of Lasky and Oxley. The examiner relies on Lasky for its teaching of an L-selectin-Ig chimeric molecule and concludes that "[w]hile applicant focuses on … Sackstein's (1997) lack of teaching of a CD44 glycoform …, Sackstein 1997 clearly [teaches] the identification of a glycoprotein L-selectin ligand expressed on the human hemopoietic cell line KG1a, wherein the L-selectin ligand does *not contain MECA antibody-specific epitopes and is not dependent on sulfation*."[18/] The examiner alleges on page 8 of the Office Action that the teachings of Sackstein 1997 may be modified by using the L-selectin-Ig of Lasky, which may be used to purify the CD44 glycoform of the present claims. This conclusion, however, ignores the teachings of Lasky and ignores the evidence of record. First, Lasky clearly uses the L-selectin-Ig to obtain *MECA-79 reactive antigen that is sulfated*.[19/] Thus, the examiner has not articulated how a "probe" used to identify sulfated, *MECA-79 reactive antigens* could be predictably used to obtain the L-selectin ligand of Sackstein 1997 that does *not contain MECA-79 antibody-specific epitopes and is not dependent on sulfation.* The examiner's own rationale is inconsistent, not reasonable, and not clearly articulated.

The Declaration of Robert Sackstein filed January 18, 2010 provided in response to Lasky (the "Lasky Declaration") explains and provides experimental evidence that L-selectin-Ig does not bind to the presently claimed glycoprotein. At paragraphs 8 - 12 of Lasky Declaration, Dr. Sackstein provides a review of the literature describing the requirement for sulfation for detection of L-selectin ligand(s) by L-selectin-Ig (*i.e.*, L-selectin-Ig detects only sulfation-dependent L-selectin ligands, and HCELL is a sulfation-independent L-selectin ligand). Accordingly, a person of ordinary skill in the art could not have combined Sackstein 1997 with Lasky to obtain the subject matter of the present invention.

The examiner also relies on Oxley for its teaching of a distinct L-selectin ligand that is expressed by KG1a cells. This reference, however, merely demonstrates that the distinct L-

---

17/ Office Action page 4, last paragraph.

18/ Office Action at page 7, last paragraph (emphasis added).

19/ See Lasky at column 4, lines 46 - 65 (Summary of the Invention)(stating that "L-selectin-IgG chimera to precipitate inorganic sulfate-labeled material" which was "abolished by treatment of the sulfate-labeled proteins with sialidase" and, further, that the "monoclonal antibody, termed MECA-79,…precipitated both components.").

selectin ligand expressed by KG1a cells is not CD34 (i.e., that it is distinct from CD34, a molecule identified by Lasky to serve as an L-selectin ligand). Accordingly, the reference represents a failure to identify and purify a distinct L-selectin ligand expressed by KG1a cells. There is no rationale provided by the examiner that would suggest that the negative results (i.e., not CD34) of Oxley would lead a person of ordinary skill in the art to the claimed subject matter.

Further, the Declaration of Robert Sackstein filed January 18, 2010 which references Oxley (the "Oxley Declaration") further explains that Oxley actually teaches away from the present invention. In the declaration, Dr. Sackstein explains in paragraphs 4 to 8 of the Oxley Declaration that the experiments disclosed in Oxley were also designed to examine a variety of different glycoproteins that could serve to support adhesion of cells. Several of these glycoproteins were well-known in the art (*e.g.,* LFA-1 (a β-2 integrin), VLA-4 (a β-1 integrin), CD44, and CD43 (a well-known sialoglycoprotein)). Table 1 of Oxley shows us that two cell lines that express CD44 (i.e., RPMI 8402 and HL60) did NOT show L-selectin ligand activity. Similarly, looking at Table 1, the only cell that displayed L-selectin ligand activity was KG1a cells, but for each molecule tested, there was a cell line that expressed at least one of the well-recognized adhesion molecule(s) but did not have any L-selectin ligand activity. Thus, it was concluded that the unidentified L-selectin ligand could not be any of the tested molecules -- NOT CD44, NOT sLex, NOT LFA-1, NOT VLA-4, NOT CD43. In Discussion (*see* Oxley at page 3305, left column, lines 17-24), Oxley concluded, specifically, the following:

> "In this study, neuraminidase-treated KG1a showed a complete loss of lymphocyte binding, indicating that sialic acid residues are also a necessary component on the KG1a L-selectin ligand; as such, lymphocyte adherence to KG1a involves carbohydrate motifs and is not based strictly on protein-protein interactions."[20/]

> "Moreover, flow cytometric analysis of the various cell lines used in the binding assay provides evidence that membrane structures such as LFA-1, VLA-4, CD44, Sialyl Lex, and CD43 do not play a primary role in lymphocyte adherence to KG1a because each of these molecules was also present on at least one other cell line tested that did not show lymphocyte binding".[21/]

[20/] Oxley beginning at page 3303, right column, first full paragraph, line 5.

[21/] Oxley at page 3305, left column, lines 17-24.

In this regard, it is clear that the findings in Oxley teach away from CD44 being the relevant scaffold for HCELL and, importantly, also teaches away that Sialyl Lex (sLex) is involved. The Oxley Declaration is submitted as evidence that one of ordinary skill in the art would not have successfully combined Oxley with Sackstein 1997 so as to conclude that the activity identified in the KG1a cells is a CD44 molecule expressing sialylated, fucosylated glycans (i.e, sLex-decorated CD44) as defined by the claims.

In view of the above, Applicant respectfully submits that the examiner has failed to clearly articulate a rationale for supporting any of the rejections set forth under 35 U.S.C. 103 and has not properly considered the evidence supporting non-obviousness of the claimed subject matter, including prior Responses to Office Actions and Declarations. An indication of allowance of all claims is solicited.

Respectfully submitted,

Dated: January 28, 2010

By: /Sheridan Snedden/
Cynthia A. Kozakiewicz, Reg. No. 42,764
Sheridan K. Snedden, Reg. No. 55,998
c/o MINTZ, LEVIN
Customer No. 30623

4699038v.1